STRATIFICATION AND BREAKING OF THE ARBACIA PUNCTULATA EGG WHEN CENTRIFUGED IN SINGLE SALT SOLUTIONS

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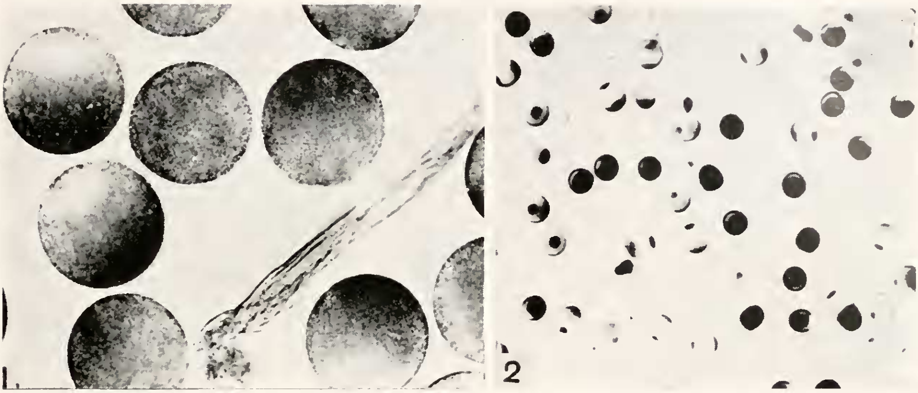
A study has been made of the comparative rate of stratification and breaking of the *Arbacia* egg in single salt solutions, when subjected to centrifugal force. It might be expected that when more rapid stratification occurs, the eggs would break apart more readily. This was, however, found not to be the case when the eggs were centrifuged in hypo- and hypertonic sea water, but this is probably due to the change in volume of the eggs (E. B. Harvey, 1943). With the increased surface area of the eggs in hypotonic sea water the tension at the surface is increased (Cole, 1932) and the eggs are more difficult to break apart. In the present experiments with pure salt solutions the surface area remained constant.

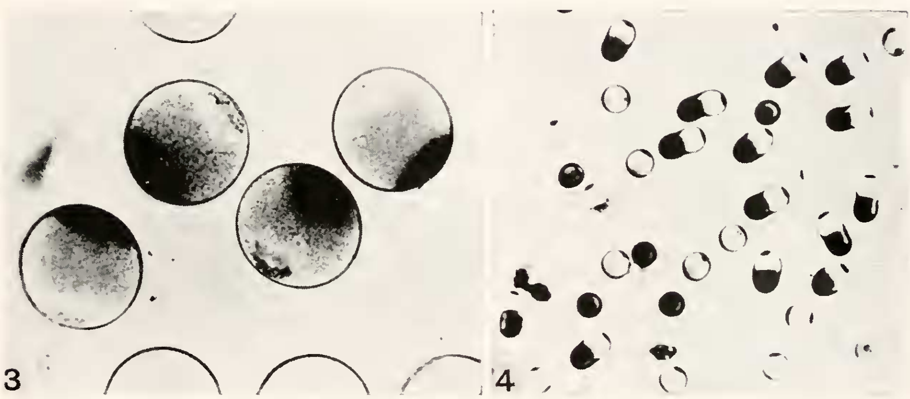
The solutions used in the following experiments were those routinely used at Woods Hole as isotonic with the sea water there, and found by me to be isosmotic on measuring the eggs after immersion, namely: 0.52 m NaCl, 0.53 m KCl, 0.34 m $CaCl_2$, and 0.37 m $MgCl_2$. The pH of the solutions was found to be respectively, 5.54, 5.44, 5.53, and 6.31. It was determined, however, that the pH in itself, at least of sea water, has no effect on the stratification and rate of breaking. Sea water was made up of pH ranging from 5 to 9 by adding HCl or NaOH; eggs kept in these solutions and centrifuged in them at the same time as those in normal sea water showed no difference in stratification or breaking. This was found also by Barth (1929) for stratification in sea water, though he did find an effect in NaCl. However Heilbrunn (1928, 1943) finds that Na definitely increases viscosity. The eggs were not injured by the pure salt solutions as they could be fertilized on removal to sea water after 40 minutes in the solutions and produced normal plutei. However, the eggs cannot be fertilized while in the solutions; the sperm are immotile in all except NaCl, and here no fertilization membrane was seen.

Arbacia punctulata eggs were placed in 50 cc. of the isosmotic salt solution for 20 minutes and this was replaced by a fresh salt solution for another 20 minutes. Three tubes of experimental eggs (in different salt solutions) and one tube of control eggs (in sea water) were centrifuged at the same time; isosmotic sugar solution was placed in the bottom of each tube to keep the eggs suspended. Care must be taken that exactly the same amount of sugar solution is used in each tube and exactly the same amount of egg suspension placed on top, so that the eggs in each tube are thrown to the same level and are subjected to exactly the same amount of centrifugal force. For stratification the force used was about 3,000 × g for two minutes, and for breaking 10,000 × g for four minutes. Each experiment was repeated many times. A single batch of eggs was always used in each experiment.

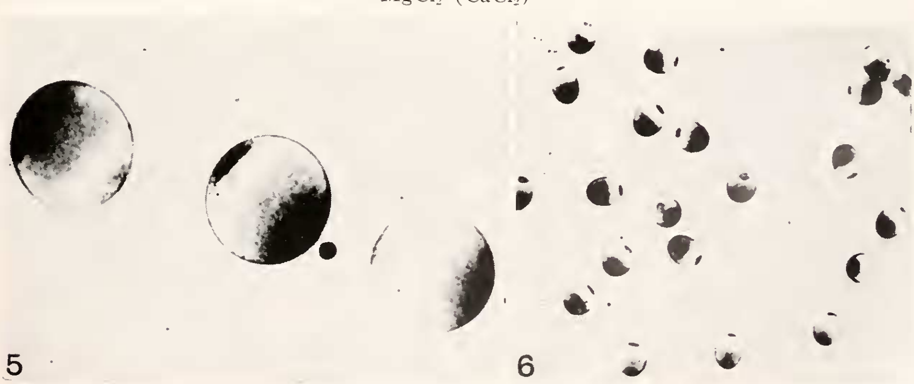
Stratification NaCl (KCl) Breaking



Sea Water



$MgCl_2$ ($CaCl_2$)



EXPLANATION OF PLATE

Stratification of *Arbacia punctulata* eggs centrifuged at 3,000 × g for two minutes in (1) NaCl, (3) sea water, (5) $MgCl_2$. Breaking apart of eggs at 10,000 × g for four minutes in (2) NaCl, (4) sea water, (6) $MgCl_2$. KCl acts much like NaCl and $CaCl_2$ much like $MgCl_2$.

The experiments were carried out at approximately 23° C., so that the temperature effect observed by Costello (1934, 1938) was not involved.

It was found that in the monovalent salts, NaCl and KCl, the rate of stratification is less than in sea water, and in the bivalent salts, $CaCl_2$ and $MgCl_2$, the rate of stratification is greater than in sea water (Photographs 1, 3, 5). The viscosity, then, is increased in NaCl and KCl and decreased in $CaCl_2$ and $MgCl_2$. In the effect on the rate of stratification the series runs, from most to least: Ca > Mg > S.W. > Na > K. This is similar to the series given by Heilbrunn (1923, 1928) in a slightly different experiment with *Arbacia* eggs, except that Na and K are reversed. This is possibly due to a difference in the tonicity of the solutions used. His series for *Stentor* is the same as my series for *Arbacia*.

In ease of breaking with centrifugal force, the series runs in the reverse order. Eggs in KCl, where the stratification is least in a given time, break most readily, and those in $CaCl_2$, where the stratification is greatest, break least readily. Eggs in the monovalent salts, NaCl and KCl, break more readily than those in sea water while the eggs in the bivalent salts, $MgCl_2$ and $CaCl_2$, break less readily than those in sea water (Photographs 2, 4, 6). In ease of breaking, the series runs, from greatest to least: K > Na > S.W. > Mg > Ca. The ease of breaking has been judged by the percentage of eggs broken in a given time with a constant force, rather than by the time for a definite percentage to break, since the experiment can be carried out more accurately when experimental and control eggs are centrifuged at the same time. An average experiment gives the following figures for percentage of eggs broken when centrifuged for four minutes at 10,000 × g.

KCl	NaCl	Sea water	$MgCl_2$	$CaCl_2$
99%	90%	50%	20%	none

There was no measurable difference in the relative size of the two "halves" in any of the pure salt solutions; the white and red "halves" were the same size as those obtained when eggs were kept and centrifuged in sea water.

There is considerable variation in ease of breaking in different lots of eggs with the same centrifugal force, and even the same batch varies slightly after being kept in sea water for several hours. In one experiment 98 per cent were broken in sea water, and 40 per cent in $CaCl_2$; in another experiment, 50 per cent were broken in NaCl and 20 per cent in sea water. In every experiment, however, the eggs in the solutions broke in the order named. It was thought that possibly the jelly surrounding the eggs might be influenced by the salt solutions and be responsible for the difference in ease of breaking. Jelly was found to be present on the eggs in all the solutions. Eggs from which the jelly was removed by addition of 0.2 cc. N/10 HCl to 50 cc. sea water, and then well washed in sea water broke in the solutions in the same order as those with jelly.

Since the experimental results are contrary to the expectation that the interior viscosity is the controlling factor in breaking of the eggs, we are led to the conclusion that the salts affect the "tension at the surface." Despite the increased interior viscosity in pure NaCl and KCl, the surface forces resisting the pulling apart of the eggs are actually decreased. In $CaCl_2$ and $MgCl_2$ they are increased though the interior viscosity is decreased. Heilbrunn (1923, 1943) has pointed out that in *Amoeba*, and apparently also in *Arbacia* eggs, the cortical protoplasm reacts differently from the interior protoplasm, and Brown (1934) has found a difference in cortical and interior protoplasm in response to hydrostatic pressure on fertilized *Arbacia* eggs.

An effect on the surface forces without any effect on the interior viscosity is given by eggs in Ca-free sea water. Unfertilized eggs kept and centrifuged in Ca-free sea water stratify at the same rate as those in sea water, as shown in previous experiments with a double image centrifuge microscope (E. B. Harvey, 1933). They break apart more readily in Ca-free sea water than in normal sea water—at about the same rate as those in NaCl alone. The fertilized eggs also break more readily in Ca-free sea water than in normal sea water, as shown previously. The absence of calcium therefore tends to decrease the surface forces and the presence of calcium alone tends to increase them. That calcium has an effect on the surface layers of eggs is well known, and has been especially emphasized by Heilbrunn (1928, 1943). A very good example is given by the classic experiments of Herbst (1900) in separating blastomeres due to the dissolution of the ectoplasmic (hyaline plasma) layer in Ca-free sea water.

Summary

When unfertilized *Arbacia punctulata* eggs are centrifuged in isosmotic single salt solutions, they stratify with decreasing readiness (indicating increasing viscosity) in the following order: $CaCl_2 > MgCl_2 >$ S.W. $>$ NaCl $>$ KCl. They break into "halves" with decreasing ease in the reverse order, those in $CaCl_2$ which stratify best, break least readily. In the bivalent salts they stratify better and break less readily than in sea water, and in the monovalent salts they stratify less and break more readily than in sea water. The ease of breaking must be determined by an effect of the salts on the surface layers rather than by their effect on the interior viscosity.

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